The Effect of Sodium Hyaluronate and Sodium Chondroitin Sulfate on the Coagulation System In Vitro

M. PANDOLFI, U. HEDNER, MD, PhD

Abstract: Healon® (sodium hyaluronate) and sodium chondroitin sulfate (CDS) are injected in the ocular cavities in a variety of operations, mainly intraocular lens (IOL) implantation. Both Healon® and CDS are structurally similar to heparin. We found that like heparin CDS has an inhibiting action on blood coagulation in vitro. The inhibiting activity is of the antithrombin type. Healon® does not possess anticoagulant activity. Since the anticoagulant effect of sodium chondroitin sulfate is observable at concentrations likely to occur in vivo the substance may impair ocular hemostasis. [Key words: anti-thrombin, chondroitin sulfate, heparin-like substance, hyaluronate, IOL implantation.] Ophthalmology 91:864–866, 1984

There is a general agreement that the introduction of Na-hyaluronate (Healon®) has implied a significant step forward in ocular surgery. Healon® is especially used to protect corneal endothelium during the introduction of intraocular lenses (IOL), but it is also employed in corneal transplantation to facilitate extracapsular cataract extraction, in glaucoma surgery to assure optimal filtration, and in retinal surgery as a vitreous substitute[1]. Recently, another viscous material, sodium chondroitin sulfate has been proposed as an agent protecting corneal endothelium.[2]

Intraocular bleeding during and after surgery is a complication which is especially annoying with IOLs. Retracting blood clots can deform the pupil and make insertion of iris-supported lenses difficult. Furthermore, blood can stain the pseudophakos and give decrease in vision.

Both Healon® and sodium chondroitin sulfate (CDS) are glycosaminoglycans whose repeating disaccharide units bear resemblance to those of the anticoagulant heparin. We investigate whether, Healon® and sodium chondroitin sulfate influence blood coagulation in vitro.

From the Department of Ophthalmology and Coagulation Laboratory, University Hospital, Malmö, Sweden.

Supported by grants from the Swedish Medical Research Council (19x-05447), Greta and Johan Kock's Foundation, The Medical Faculty, University of Lund and Pharmacia AB, Uppsala, Sweden.

Reprint requests to U. Hedner, MD, PhD, Assoc. Prof., Head of the Department of Coagulation Disorders, University Hospital, 214 01 Malmö, Sweden.

MATERIALS AND METHODS

Healon® and sodium chondroitin sulfate were obtained from the producers (Pharmacia and Cilco Inc.) in syringes devised for immediate surgical application. The concentrations of sodium chondroitin sulfate and Healon® intended for intraocular use were 500 mg/ml and 10 mg/ml, respectively. The substances were stored at 4°C.

FIBRINOLYTIC TESTS

Fibrinolytic activity was measured with a modified fibrin plate method.[3] According to this method, the samples to be tested are applied on a fibrin film, which is made by letting plasminogen and human fibrin (Kabi Vitrum) clot with bovine thrombin (Topastasin®, Hoffman-La Roche. After incubation of the plates in 37° C for 18 hours, lysis zones are formed. The areas of the lysis zones are proportional to the fibrinolytic activity of the sample and are measured in mm^2. Serial dilution (1/1, 1/2, 1/4, 1/8, 1/16) with unbuffered 0.15 M NaCl were made of both Healon® and sodium chondroitin sulfate, and

Table 1. Fibrinolytic Activity of Healon®

	Concentration					
	1:1	1:2	1:4	1:8	1:16	1:32
H alone	0	0	traces	0	0	0
H + urokinase	265*	273	295	269	297	300
H + cornea activator	700*	670	672	685	675	696
H + porcine activator	250*	268	296	308	255	270

H = Healon®

* Activity of mixtures of activators and buffer.

Serial dilutions of Healon® mixed equal parts of activator and tested on fibrin plates. Fibrinolytic activity expressed in mm^2 of lysis.

incubated at room temperature for 3 minutes with urokinase Leo 50 PU/ml, with plasminogen activator collected from in vitro culture of rabbit cornea[4] or with tissue plasminogen activator extracted from a pig's uterus (gift of Preben Kok, Umeå).

COAGULATION TESTS

Serial dilutions (1/8, 1/40, 1/160, 1/1280) with Tris buffer (Tris chloride 75 mM, pH 7.8) were made of both Healon® and sodium chondroitin sulfate. For control, similar dilutions of 0.15 M NaCl were made. Recalcification time (plasma coagulation time after addition of Ca^{2+}), thrombin time, Reptilase time, one-stage prothrombin time (plasma coagulation time after addition of thromboplastin reflecting the second phase of coagulation), P & P (factors II, VII, X activity) factor V activity, were determined according to procedures described earlier.[5,6] Factor VIII coagulant activity (VIII:C) and factor IX coagulant activity (IX:C) were determined with a one-stage assay using platelet-rich hemophilia A/B plasma as test base.[5,6] Factor VIII related antigen (VIII R:Ag) was assayed immunochemically using the rocket technique as described by Holmberg and Nilsson (1973).[7] Antithrombin III was assayed by electroimmunoassay,[8] as well as amidolytically.[9] Factor XIII (fibrin stabilizing factor) was assayed using the "activity staining technique" described by Henrikson et al (1979).[10] The thrombin time and Reptilase time were described in detail by Nilsson et al (1982).[11]

RESULTS

FIBRINOLYTIC STUDIES

Healon® did not show any fibrinolytic activity on plasminogen-rich fibrin plates at any concentration (Table 1). Nor did Healon® show any inhibitory or potentiating effect on the fibrinolytic activity of urokinase, cornea activator or porcine plasminogen activator. Similar results were obtained with sodium chondroitin sulfate.

COAGULATION STUDIES

We found that sodium chondroitin sulfate prolonged the recalcification time (Table 2) of citrate plasma even at dilutions as large as 1/160 (corresponding to a final concentration of 3 mg/ml). Healon® had no such effect. The one-stage prothrombin time was prolonged (Table 2) by sodium chondroitin sulfate (≥1/40; 13 mg/ml). Healon® did not prolong the one-stage prothrombin time until present in a concentration of 1/8 (≥0.3 mg/ml). The prothrombin complex (prothrombin, proconvertin, factor X) activity was clearly inhibited by sodium chondroitin sulfate at concentrations of 1:8 (63 mg/ml) and so was factor VIII coagulant (VIII:C) and factor V activity. No such inhibition was ever found in the presence of Healon.® The thrombin time (Table 3) was shortened by Healon® and prolonged by sodium chondroitin sulfate. No corresponding effect on the Reptilase® time was seen indicating that the anticoagulant effect of sodium chondroitin sulfate cannot be ascribed to any direct interference with fibrinogen. Table 4 shows the action of Heparin® on the same system. No effect on the factor VIII, fibrinogen or ATIII was found from either Healon® or sodium chondroitin sulfate.

DISCUSSION

The results show that CDS has a clotting inhibitory effect which seems to be the consequence of a heparin-like antithrombin action. An anticoagulant activity in vitro by CDS was also found by Bjornsson et al (1982).[12] Recently the anti-thrombin effect and the inhibitory effect

Table 2. Coagulation Activity of NaCl, Healon,® and Sodium Chondroitin Sulfate

	Concentration											
	NaCl				Healon®				Sodium Chondroitin Sulfate			
	1:8	1:40	1:160	1:1280	1:8	1:40	1:160	1:1280	1:8	1:40	1:160	1:1280
Recalcification of citrated plasma time (seconds)	100	98	106	99	120	101	98	96	>10 min	300	185	120
AHF (factor VIII)/VIII:C %	158	153	205	198	113	117	165	173	<2.5	125	150	120
One-stage prothrombin time (seconds)	16.1	15.6	15.7	15.8	19	16.2	15.8	15.8	30.5	18.6	16.8	16.8

AHF = anti-hemophilic factor; C = coagulant.

Table 3. Effect of Healon® and Sodium Chondroitin Sulfate on Thrombin Time*

Concentration	Clotting Time Seconds	
	Healon® 10 mg/ml	Sodium Chondroitin Sulfate
Buffer	41	41
1:4	24	120
1:8	23	91
1:10	30	99
1:40	35	95
1:80	40	74
1:160	37	59
1:320	39	65
1:640	44	55

*Citrate human plasma.

Table 4. Effect of Heparin® on Thrombin Time

Concentration (I.U.)	Clotting Time (seconds)
buffer	47
0.008	>180
0.006	>180
0.004	85
0.002	54
0.001	46

IU = intravenous units.

on activated factor X (Xa) of the heparin molecule have been located to different parts of the molecular structure. Thus low molecular weight heparins have been found to have almost no anti-thrombin activity but still possess a substantial inhibitory effect on Xa.[13,14] Such heparins do not cause an increased bleeding tendency.[15] In addition, heparin-like substances having less anti-coagulant activity (as measured in an APTT, activated partial thromboplastic time system) than natural heparin are described.[15] The substances studied are two different glycosaminoglycans with a structure similar to that of heparin. One of them (CDS) turned out to behave like heparin in the coagulation assays thus having a substantial anti-thrombin effect (3.2 mg/ml corresponding to a heparin concentration of 0.004 IE/ml). This action is well in agreement with other uses of CDS which, like heparin, has a antithrombotic-anti-arteriosclerotic effect in man and experimental animals.[16,17,18] The anticoagulant effect of CDS is present at concentrations which are likely to occur in a closed little cavity such as the anterior segment of the eye. In this case CDS should have an unfavorable effect on the local hemostasis resulting in a prolongation of hemorrhages from the section of from the iris. However, Healon® turned out to have almost no anti-thrombin activity in vitro in concentrations of up to 0.6 mg/ml (final concentration). On the contrary a shortening of the thrombin time was observed in concentrations of 0.6 to 0.07 mg/ml (1:4, 1:8, 1:10, 1:20, 1:40) at least in the presence of 0.7 IU/ml thrombin. Both substances in the vitro assays were used in concentrations recommended for clinical use. Thus, sodium chondroitin sulfate is used in doses several hundred times higher than those recommended for Healon.® It could be concluded that in the concentrations used in clinical practice, Healon® does not inhibit coagulation, nor does it interact with the fibrinolytic system. This substance should be the most favourable in ophthalmological practice.

REFERENCES

1. Miller D, Stegmann R. Healon; A Comprehensive Guide To Its Use In Ophthalmic Surgery. New York: Wiley & Sons, 1983.
2. Soll DB, Harrison SE, Arturi FC, Clinch T. Evaluation and protection of corneal endothelium. Am Intra-Ocul Implant Soc J 1980; 6:239–42.
3. Nilsson IM, Olow B. Fibrinolysis induced by streptokinase in man. Acta Chir Scand 1962; 123:247–66.
4. Pandolfi M, Lantz E. Partal purification and characterization of keratokinase, the fibrinolytic activator of the cornea. Exp Eye Res 1979; 29:563–71.
5. Nilsson IM, Blombäck M, Ramgren O. Haemophilia in Sweden. I. Coagulation studies. Acta Medi Scandi 1961; 170:665–82.
6. Nilsson IM. Haemorrhagic and Thrombotic Diseases. London: John Wiley & Sons, 1974.
7. Holmberg L, Nilsson IM. Immunologic studies in haemophilia A. Scand J Haematol 1973; 10:12–6.
8. Hedner U, Nilsson IM. Antithrombin III in a clinical material. Thromb Res 1973; 3:631–41; Also: Bibl Anat 1973; 12:267–71.
9. Ødegård OR, Lie M, Abildgaard U. Heparin cofactor activity measured with an amidolytic method. Thromb Res 1975; 6:287–94.
10. Henriksson P, Stenberg P, Nilsson IM, et al. A specific, fluorescent activity staining procedure applied to plasma and red blood cells in congenital factor XIII deficiency. Br J Haematol 1980; 44:141–7.
11. Nilsson IM, Rothman U, Stenberg P, et al. A novel semi-synthetic sulphated polysaccharide with potent antithrombin activity. Br J Haematol 1982; 50:335–43.
12. Bjornsson TD, Nash PV, Schaten R. The anticoagulant effect of chondroitin-4-sulfate. Thromb Res 1982; 27:16–21.
13. Laurent TC, Tengblad A, Thunberg L, et al. The molecular-weight-dependence of the anti-coagulant activity of heparin. Biochem J 1978; 175:691–701.
14. Andersson LO, Barrowcliffe TW, Holmer E, et al. Molecular weight dependency of the heparin potentiated inhibition of thrombin and activated factor X. Effect of heparin neutralization in plasma. Thromb Res 1979; 15:531–41.
15. Esquivel CO, Bergqvist D, Björck C-G, Nilsson B. Comparison between commercial heparin, low molecular weight heparin and pentosan polysulphate on hemostasis and platelets in vivo. Thromb Res 1982; 28:389–99.
16. Meuleman DG, Hobbelen PMJ, von Dedem G, Moelker HCT. A novel anti-thrombotic heparinoid (Org 10172) devoid of bleeding inducing capacity: a survey of its pharmacological properties in experimental animal models. Thromb Res 1982; 27:353–63.
17. Nakazawa K, Murata K, Izuka K, Oshima Y. The short-term effects of chondroitin sulfates A and C on coronary atherosclerosis subjects with reference to its anti-thrombogenic activities. Jpn Heart J 1969; 10:289–96.
18. Robbins RC, Morrison LM, Simpson CF. Effect of chondroitin sulfate A and flavinoids on hypervitaminosis D in rats. Proc Soc Exp Biol Med 1969; 131:719–22.
19. Morrison LM, Bajwa GS, Hernandez HJ, Ershoff BH. Increased coronary collateral circulation in monkeys and rats with coronary heart disease by chondroitin sulfate A. Abstract. Circulation 1971; 44(Suppl 2):II–23.